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Stability indicating HPLC method development and validation of Fostemsavir in bulk and marketed formulations by implementing QbD approach

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Abstract: Achieving a predictable degree of quality with intended and planned specifications is known as quality by design (Quality-by-Design). QbD is an alternative to conventional method development that places more attention on identifying and mitigating potential risks. Component of the Quality-by-Design methodology involves conducting a series of experiments to learn how various factors, including the dependent variables, affect the answers of interest. Here, we use a QbD loom to detail the creation and verification of a stability-indicating high-performance liquid chromatography (HPLC) method for Fostemsavir in both bulk and finished-goods forms. This work presents a workable experimental design for optimising the RP-HPLC separation technique by identifying the optimum mobile phase concentration and flow rate. Here, we propose a practical experimental layout for determining the RP-HPLC separation technique's optimal mobile phase concentration and flow rate. Using Design Expert version 13.0, the optimum chromatographic conditions were determined to be as-Shim-pack GIST C18 (250 mm × 4.6 mm × 5.0 µm), mobile phase acetonitrile to 1% formic acid (80:20, v/v), flow rate 0.8 ml/min, and retention period 3.24 min. At a detection wavelength of 266 nm, it was discovered that the devised technique was linear over a concentration range of 50-90 µg/ml (r^2 = 0.997). Test parameters for the system's appropriateness were determined to be 1.124 for the tailing factor and 9480 for the theoretical plates. Intraday RSD was found to range from 0.70 to 0.94, whereas interday RSD was found to range from 0.55 to 0.95 percent. Values for robustness were under 2%. The solution stability % RSD was calculated to be 0.83. The result of the assay was 100.05 percent. The created methodologies were used for studies of forced degradation, and the stressed materials were analysed. The parameters used to validate the procedure fell within the acceptable range recommended by ICH. Using Design Expert 13.0, we created a central composite design experiment that illustrates the relationships between the mobile phase and flow rate across three levels, with retention duration, tailing factor, as well as theoretical plates as the responses of interest. By this work, we gain insight into the variables that affect chromatographic separation and strengthen our conviction that HPLC method will serve industrial needs. Quantitative method development was applied to improve comprehension of multi-tiered method variables.

Introduction

Fostemsavir was approved in 2020 by USFDA. Fostemsavir chemically, {3-[(4-Benzoyl-1-piperazinyl) (oxo) acetyl]-4-methoxy-7-(3-methyl-1H-1, 2, 4-triazol-1-yl)-1H-pyrrolo[2, 3-c] pyridin-1-yl} methyl dihydrogen phosphate. The molecular weight of Fostemsavir is 583.498 g.mol^{-1}, and its chemical formula is $C_{37}H_{48}N_4O_5$. HIV entry inhibitor fostemsavir is a temsirolimus prodrug

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(BMS-626529). The gp120 protein is the focus of fostemsavir, an HIV-1 attachment inhibitor (Gorycki et al., 2022) (Fig 1).

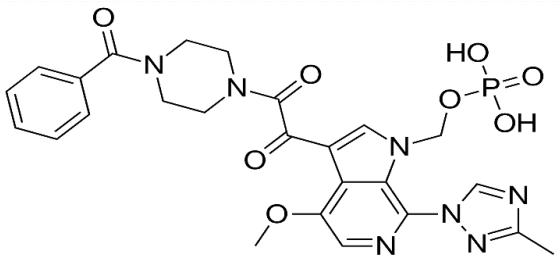

Figure 1. Structure of Fostemsavir

Although it dissolves in DMSO, methanol, and acetonitrile, water is not a good solvent for fostemsavir. The Indian Pharmacopeia has approved fostemsavir for use. As per ICH, the drug stability test indicates guidelines (Q1A); since the tests used to test stability samples should be stability indicated, their results should be fully confirmed (Guideline, 2003).

The literature reveals various methods of development of the HPLC of Fostemsavir. Fostemsavir and ganciclovir were estimated and validated in a new way using RP-HPLC (Lamichhane et al., 2022).Novel HIV-1 entry inhibitors were characterised kinetically, and an association between off-rate and potency was found (Meuser et al., 2018).The study also shows that an LCT-MS/MS approach was successfully developed and validated for measuring temsavir plasma concentrations in HIV patients (Thoueille et al., 2023). Analyzing the Effects of Forced Degradation on Antiretroviral Drugs Authorized by the FDA (Lamichhane et al., 2022).The surprising resistance to enzymatic hydrolysis demonstrated by a prodrug encapsulated in a hydrated HPMC matrix tablet is also described (Hanley et al., 2022).It has been revealed that the metabolic stability of novel HIV-1 entry inhibitors is affected by their core region's composition and orientation (Karadsheh et al., 2020).The Kinetic and Pharmacokinetic Characteristics of a Redesigned HIV-1 Entry Inhibitor Core Region (Karadsheh et al., 2021)The *in-vitro* antiviral activity of a *Cistus incanus* extract is quite potent, blocking the formation of viral envelope proteins and thus working against HIV and Filoviruses (Rebensburg et al., 2016).

QbD is an approach to development that emphasises considering and controlling each step of the production process, with the assistance of scientific principles as well as quality risk management, to set clear goals from the get-go (Roy, 2012). Regulatory bodies frequently highlight quality guidelines from the International Council for Harmonization (ICH). These standards include Q8 Pharmaceutical Development, Q9 Quality Risk Management, and Q10 Pharmaceutical Quality System (ICH, 2005; ICH, 2008; ICH, 2009; Borman et al., 2007).Instead of testing techniques for quality after they have been developed, QbD principles emphasise incorporating quality into the method throughout development. Knowledge of factor and interaction effects by a selected set of research is a potent tool that may be accessed with QbD. To guarantee that the method continues to work throughout the life of the product, quality by design (QbD) for HPLC procedures requires early evaluation of robustness and ruggedness. Adapting a system that is not strong or resilient can waste a lot of time and resources on things like redeveloping, revalidating, and retransferring analytical procedures. Failure mode identification and defining a reliable method of operational design region and design space have been the key focuses of a QbD within the context of applicable system suitability criteria and ongoing life cycle management (Schweitzer et al., 2010; Galen, 2004; Snyder et al., 1997; Bhatt et al., 2011; Rajkotwala et al., 2016; Singh et al., 2017).

The literature review uncovered reports of QbD strategies using the HPLC method. It has been reported that QbD-based pro-liposome generation of Fostemsavir for enhanced oral bioavailability has been successful (Patel et al., 2009). Fostemsavir oral bioavailability and lymphatic intestinal absorption enhancement using the quantum-based design of solid self-nano emulsifying oily formulations (S-SNEOFs) (Dhand et al., 2020).

Materials and Methods

Drug samples

Tablets used: Brand: RUKOBIA; Fostemsavir 600mg. Manufactured by: ViiV Healthcare.

Chemicals and solvents: We only ever utilised HPLC-grade solvents and reagents. Water, Methanol, Acetonitrile, 1% Formic Acid, and Distilled water.

Chromatographic instrumentation:

The HPLC system (LC-2030C Plus; Shimadzu, Kyoto, Japan) was implemented to improve procedures. The temperature-controlled column compartment of the high-performance liquid chromatography (HPLC) equipment, LC10AT quaternary solvent pump, an auto sampler and an Ultra violet (UV) wavelength detector.The micro vortex mixture (foure's scientific, New York, USA) and the LC solution software (Liquid Chromatography Solution) were used to collect, analyse, and report the data. For the HPLC analysis, we utilised a Shim-pack GIST C18 (Shimadzu ®) column with a 5 µm particle size, 4.6 mm internal diameter, and 250 mm length.

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Chromatographic conditions

We used a Shim-pack GIST C18 column [250 mm× 4.6 mm, 5 µm particle size, equilibrated in acetonitrile to 1% Formic Acid (80:20 v/v)]. The column was maintained at room temperature with a 0.8 ml/min flow rate. A 266.0 nm UV detector was used to monitor the eluents. A central composite design optimised the Fostemsavir HPLC process for mobile phase and flow rate.

Preparation of standard solution

A 1000 µg/ml standard stock solution of fostemsavir was prepared by dissolving 10 mg/ml of the drug into the mobile phase. Diluting the stock solution yielded 100 µg/ml using the mobile phase to bring the volume up to 10 ml and 1 ml of the sub-stock solution was diluted to yield the 10 µg/ml solution.

Selection of detection wavelength

10 µg/ml, the optimal detection wavelength for fostemsavir was found to be at 266 nm after scanning between 200 and 400 nm.

HPLC method development by QbD approach

Analytical QbD's approach to HPLC method development was as follows.

Selection of quality target product profile

The QTPP is useful for pinpointing the factors that impact the QTPP settings. With the proposed HPLC method, we determined the QTPP to be the retention duration, tailing factor, and theoretical plates (Turpin et al., 2008).

Determine critical quality attributes

The QTPP's CQAs are most essential. QTPP response range was maintained by controlling mobile phase composition and flow rate (Myers et al., 2016).

Factorial design

The mobile phase, as well as the flow rate of the HPLC technique, were optimised and selected using the central composite experimental design based on the definition of the QTPP and CQAs. By employing a central composite statistical screening methodology, we analysed the impacts of mobile phase composition and flow rate on retention duration, tailing factor, and theoretical plates, including their interaction and quadratic nature. Design Expert® (Version 13.0, Stat-Ease Inc. and M M) was used to find the ideal design for a two-factor, mobile phase composition with flow rate at three distinct levels, examining quadratic response surfaces for a second-order polynomial.

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{12} AB + \beta_{11} A_2 + \beta_{22} B_2 B + \beta_{22} B_2 A + \beta_{11} A_2$$

In the case of level-coded factors A and B, Y is the measured response for each level combination, 0 is an intercept, and 1 through 22 are regression coefficients generated from studies evaluating the observed experimental values of Y. ABC and A2B2 show quadratic term interaction. The elements were selected through exploratory research into the complex interplay of variables and process parameters (Yubing, 2011). Independent variables, including mobile phase composition and flow rate, are shown in Table 1. The proposed independent variables were most interested in retention time, tailing factor, and theoretical plate (Krull et al., 2009).

Evaluation of experimental results and selection of final method conditions

We employed the CCD technique to assess these methodological factors. Initially, we examined the retention time, tailing factor, and theoretical plates in several contexts. This resulted in unique chromatographic settings for Fostemsavir. Changes to the method's parameters don't significantly affect quality in the demonstrated acceptable ranges. This prevents problems with the method from showing up during validation later on. Several iterations of tuning the variable are required before the results of the modelling studies can be considered satisfactory (Reid et al., 2013). The Design Expert software will be used to find the optimal chromatographic settings.

Table 1. Coded values for independent variables

Level of Variable	Factor	
	Mobile Phase Composition % (ACN: 1% Formic Acid)	Flow Rate (mL/min)
Low Level (-1)	70:30	0.8
Medium Level (0)	80:20	1.0
High Level (1)	90:10	1.2

Risk assessment

All of the method's qualities, like its efficiency and durability, are considered to determine how best to optimise the final technique. The ICH Q8 and ICH Q9 QbD guidelines used a risk-based methodology to evaluate the method's robustness and ruggedness (Molnar et al., 2010). The characteristics and performance of the method were subjected to robustness and ruggedness tests, which were conducted in many different labs, with different chemicals, analysts, tools and reagents, and then on different days (Monks et al., 2012; Ramalingam et al., 2015; ICH, 2012; Orlandini et al., 2013).

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Implement a control strategy

After a method has been developed, it is time to implement a strategy for maintaining control. Analytical controls were developed when the target profile was established. Analytical control strategy refers to the predetermined set of checks and balances established in light of the knowledge of the many parameters, such as purposefulness, methodology, and risk. They verify the method's efficiency and results fit analytical expectations. The sample making, measuring, and controlling processes were subject to an analytical control strategy (ICH, 2012).

Continual improvement for managing analytical life cycle

Monitoring quality consistency and performing routine continuation and software updates on HPLC instruments and computers, with supporting instrumentation and apparatus in-house, is the ideal method for managing the analysis lifecycle (Orlandini et al., 2013).

Analytical method validation

A method is considered validated when sufficient documentation exists to support the claim that it was utilised to confirm an appropriate analytical process. Fostemsavir's HPLC technique development and validation followed ICH Q2 (R1) regulations (ICH, 2005).

Linearity

Fostemsavir's linearity was tested across a 50-90 µg/ml concentration range by assessing 5 replicate levels. Peak area (y-axis) versus concentration (x-axis) were plotted to generate the calibration curve. The regression line equation and values of the correlation coefficient were computed.

Accuracy

Recovery studies from commercially available formulations were used to calculate the method's precision at 50%, 100%, and 150% of the standard addition. Fostemsavir recoupment percentages were determined. Recovery rates between 98% and 102% of the reference addition were considered acceptable in the ICH guidelines.

Precision

Six 50 µg/ml Fostemsavir samples were measured for accuracy. Fostemsavir concentrations of 50 µg/ml, 60 µg/ml, and 70 µg/ml were analysed three times on the same day, separated by 2 hours, and on three separate days, to establish intraday and interday precision. Lower than 2% RSD was considered acceptable.

Ruggedness

The results should be evaluated for robustness concerning the variation introduced by external factors such as analysts, laboratory, equipment, reagents, and days. This proposed approach shifted the focus of toughness research away from the analyst as a potential confounding variable. The computed % RSD of the peak area was below 2, the threshold for approval.

Robustness

Adjusting the mobile phase ratio, pump flow rate, column temperature, and wavelength assessed the procedure's resilience.

Effect of variation in Flow rate

The results of an investigation into the consequences of fluctuating flow rates are presented. Three different concentrations of the test solution were produced and injected into the HPLC system at flow rates of 0.6 ml/min and 1.0 ml/min in accordance with the test method. The test procedure was used to assess the parameters of the system's appropriateness.

Effect of Variation in Wavelength

The impact of wavelength shift was investigated in this study. Three different concentrations of the test solution were injected into the HPLC system and analysed at 265 nm and 267 nm according to the test method. The test method was used to assess the parameters of the system's appropriateness.

Effect of Variation in Temperature

Researchers looked at the impact of varying column temperatures. Following the protocol, three different test solution concentrations were injected into the HPLC system and subjected to temperatures of 35°C and 45°C in the column. The test procedure was used to assess the parameters of the system's appropriateness.

Effect of variation in Ratio of Mobile Phase

Results from experimenting with different values for the Ratio of Mobile Phase were studied. The test solution was produced in three doses and put into the HPLC apparatus using a 70:30 or 90:10 acetonitrile: formic acid mobile phase. The system's parameters' correctness was determined using the test technique.

LOD and LOQ

A drug's detection limit (LOD) is the concentration at which it can be clearly distinguished from background levels. In contrast, the quantification limit (LOQ) is the concentration at which it can be measured with statistical significance. In order to calculate LOD and LOQ in accordance with ICH recommendations, the following equation was utilised.

$$LOD = 3.3 (\sigma/SD)$$

$$LOQ = 10 (\sigma/SD)$$

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Here, SD represents the calibration curve's slope and the regression line's y-intercept standard deviation.

System suitability studies

Six independent analyses of Fostemsavir were performed to determine the system's viability. Standard solutions' retention times, tailing factors, column efficiencies, peak asymmetries, and theoretical plates were determined.

Solution stability

Fostemsavir's solution stability was tested by letting the standard solution sit in a volumetric flask with a tight-fitting cover for 48 hours at room temperature. After 24 and 48 hours, we first measured the medications' peak regions.

Forced degradation study

Acid induced-degradation

The volume of a 100 ml volumetric flask was adjusted by adding more mobile phase after 10 mg of Fostemsavir and 50 ml of mobile phase was added to it and sonicated for 20 minutes. Using 1 ml of solution from this stock solution added to a 20 ml volumetric flask, followed by 10 ml of mobile phase, the samples were strained at 60°C in a water bath for 8 hours, cooled to room temperature, and neutralised of acid with the base of equivalent concentration and volume. Fill in the void with the mobile phase and stir. Use the filtrate after filtering the solution through a 0.45 membrane. After that, an improved chromatographic system was used to chromatograph these filtrates.

Base induced-degradation

1 ml of 1N NaOH was added to the fostemsavir solution, and the samples were heated to 60°C for eight hours before being cooled to room temperature in a water bath. The final concentration of fostemsavir in these solutions was adjusted to 10 µg/ml by diluting it with the mobile phase. Before further use, the solution should be filtered via a 0.45 micron membrane filter. These filtrates were then analysed by chromatography in a highly refined chromatographic environment.

Hydrogen Peroxide- induced degradation

The fostemsavir solution was oxidised with 1 ml of hydrogen peroxide (3% w/v). For 24 hours, these solutions were stored at room temperature out of direct sunlight. The concentration of Fostemsavir in these solutions was adjusted to 10 µg/ml by diluting them with the mobile phase. Use the filtrate after filtering the solution through a 0.45 µ membrane. After that, an improved chromatographic system was used to chromatograph these filtrates.

Thermal degradation

Fostemsavir samples (10 mg) were dissolved in Methanol and diluted with water to reach a final volume of 10 ml before being heated in an oven at 50°C for 3 hours. After making the necessary dilution with the mobile phase, the solution was injected under stable chromatographic conditions.

Light heat degradation product

Fostemsavir (10 mg) was dissolved in methanol (10 ml). During 8 hours, the solution was left out in the sun. The aforementioned solution was withdrawn at 1 mL and further diluted with methanol to 10 mL. Diluting the solution with the mobile phase decreased the concentration to 10 µg/ml. The material was injected into the column to create the chromatograms.

Assay

The average weight of twenty tablets was established by precise weighing. The tablets were crushed even more, and the resulting powder was added to 100 ml of methanol to represent 100 mg of Fostemsavir. The solution was concentrated to 200 µg/ml by transferring 2 ml to a 10 ml volumetric flask and passing it through a 0.45 µ membrane filter. HPLC analysed the solution, and the conclusions were linear. For this computation, we averaged results from three separate tests.

Result

An 85:15 volume/volume (v/v) methanol to mobile water phase was first attempted, and while the peak was seen, the theoretical plate count was below the USP standard. It was attempted to use an 80:20 volume/volume (v/v) acetonitrile to water the mobile phase, but the tailing factor was poor. Theoretical plate yield and tailing factor were not maximised using a mobile phase of 70% acetonitrile, 20% methanol, and 10% formic acid. The second mobile phase that was tried was acetonitrile:1% formic acid (70:30 v/v). The peak shape has improved, however, there are fewer theoretical plates than USP requires. The chromatographic conditions needed to pass the system suitability test were met. The ideal mobile phase is 80 percent acetonitrile and 20 percent formic acid (v/v). Several design space characteristics were optimised concerning the central composite design.

HPLC method development by QbD approach

Quality target product profile

Retention duration, tailing factor, and theoretical plates were selected as the QTPP for optimising HPLC chromatographic settings (Reid et al., 2013).

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Table 2. Fostemsavir optimization of parameters using CCD

Run	Factor-1 ACN to 1% Formic Acid	Factor-1 Flow Rate	Response-1 Retention Time	Response-2 Tailing Factor	Response-3 Theoretical Plate
1	70:30	0.8	4.329	1.120	7984
2	80:20	1.0	3.261	1.129	7894
3	90:10	1.2	2.640	1.130	8892
4	80:20	1.0	3.290	1.126	7841
5	70:30	1.0	3.268	1.129	7991
6	90:10	1.0	3.261	1.127	8782
7	80:20	1.2	2.640	1.128	7850
8	80:20	1.0	3.268	1.127	7908
9	70:30	1.2	2.640	1.128	7990
10	80:20	0.8	3.243	1.124	9486
11	90:10	0.8	3.261	1.127	8613

Table 3. Obtained solution for optimized formulation

Code	ACN to 1% Formic Acid	Flow Rate	Retention Time	Tailing Factor	Theoretical Plate	Desirability
C10	80:20	0.8	3.243	1.124	9486	1.0

Critical quality attributes

Acetonitrile (80%) and formic acid (20%) were discovered to be the main components of the mobile phase.

Factorial design

The proposed HPLC method will be developed using the central composite architecture. Parameter optimisation results are displayed in Table 2 (Elder, 2013).

Design space

The research included a central composite design, a quadratic design layout, and 11 response surface study type iterations. Retention time, tailing factor, and theoretical plates were analysed in connection with mobile phase composition and flow rate using the proposed CCD experimental design.

According to the information in Figure 2 and the equation for retention time (calculated from actual values), 56.75 + 0.028 × A -19.01× B - 0.010 × AB + 0.000343 × A2 + 1.70458 × B2, it was found that the retention time increased in direct proportion to the rise in the amount of acetonitrile in the mobile phase (A), as indicated by the positive coefficient of 0.028 (β1). On the other hand, as the flow rate (B) increased, the value of retention time dropped, as shown by the negative coefficient of -19.01 (β2).

The value of theoretical plates was found to rise based on the data in Figure 3 and the theoretical equation for plates (derived from actual values), i.e., − 16774.36 − 4220.40 × A + 53225.20 × B + 56.05 × A × B + 26.83 × A2 − 4380.60 × B2. This is most likely because of the negative coefficient of β1 (-4220.40), which shows that an increase in theoretical plates would result from a decrease in the amount of acetonitrile in the mobile phase (A). A rise in theoretical plates would also be predicted by an increase in the pH of buffer (B), according to the positive coefficient of β2 (53225.20).

In accordance with the data shown in Figure 4 and the peak asymmetry equation (derived from actual values), which is 31.13 − 0.31 × A − 5.98 × B + 0.0055 × A × B + 0.0021 × A2 + 0.429 × B2, it was found that the peak asymmetry's magnitude increased in direct proportion to the negative coefficient of β1 (-0.31). This suggests that it is best to reduce the amount of acetonitrile in the mobile phase (A). Additionally, two negative coefficients (-5.98) were found, showing that a rise in peak asymmetry would also be caused by a fall in the buffer's pH (B).

Optimized condition obtained

Table 3 provides the optimal HPLC conditions and predicted responses, using Design expert 13.0 to analyse all replies under different trial conditions. We conducted the HPLC chromatogram for a specific mobile phase and flow rate and compared the observed responses to the expected values to calculate the percentage prediction error.

Method validation

System suitability

System compatibility was evaluated by analysing a sample chromatogram across a range of measures, including retention time (3.243 min), theoretical plates (9486), tailing factor (1.124), and relative standard deviation (RSD) of six replicate injections (0.154). A 3D

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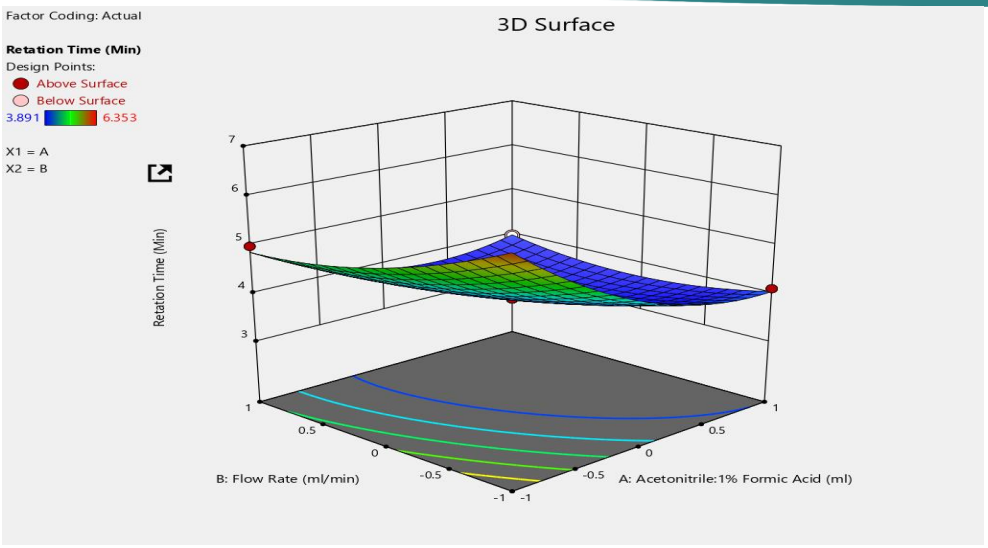

Figure 2. 3D surface plot of Fostemsavir by using central composite design for the effect of the combination of factors on retention time

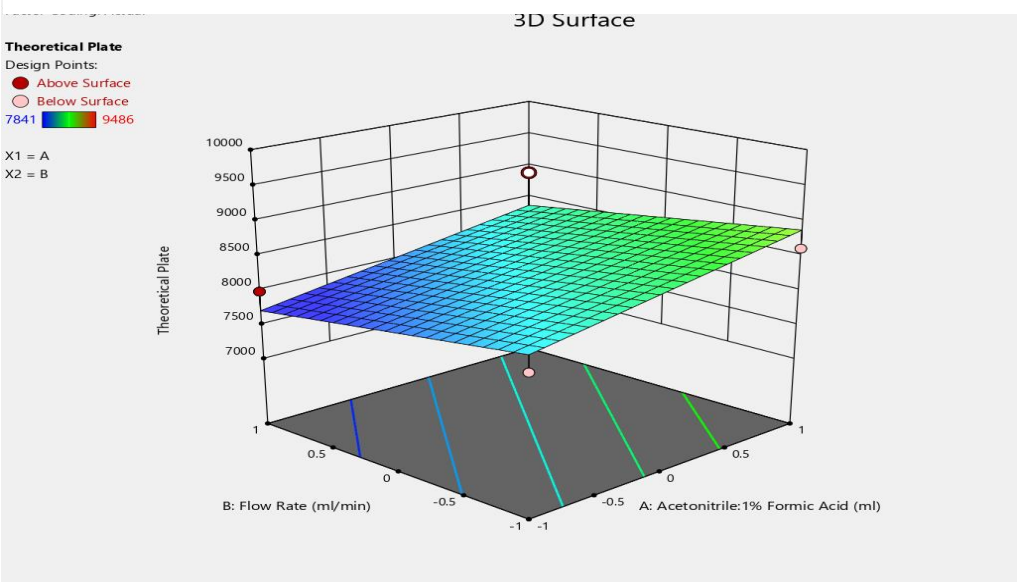

Figure 3. 3D surface plot of Fostemsavir by using central composite design for the effect of the combination of factors on theoretical plate

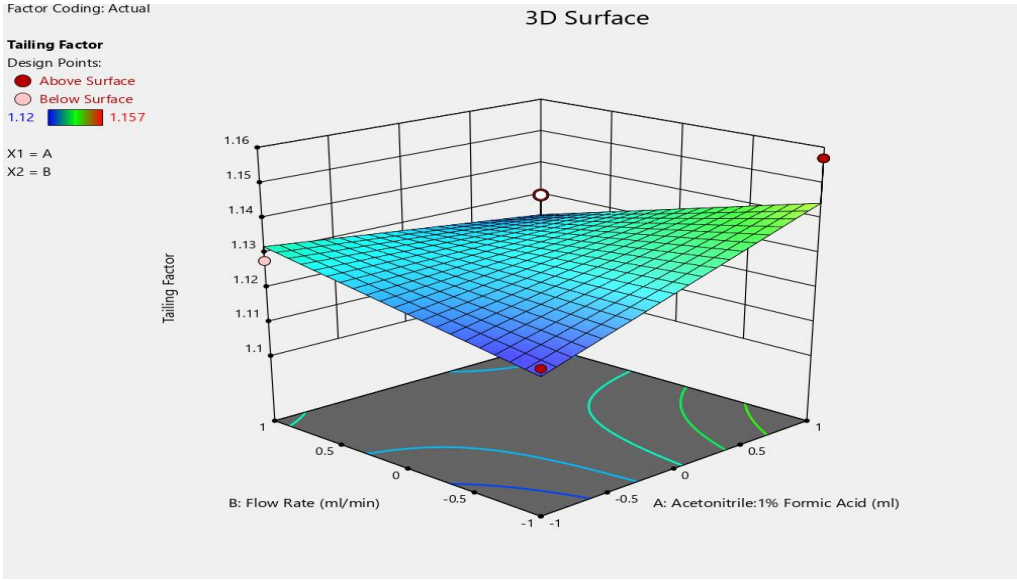

Figure 4. 3D surface plot of Fostemsavir by using central composite design for the effect of the combination of factors on the tailing factor

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Table 4. Linearity of Fostemsavir

Sr. No.	Concentration	Peak Area	SD	Correlation coefficient (r^2)
1	50	1738690	538484.7	0.997
2	60	2246107		
3	70	2662516		
4	80	2900682		
5	90	3076136		

Table 5. Repeatability of Fostemsavir

Sr. No.	Concentration µg/ml	Peak Area	Mean	SD	%RSD
1	50	1739161	1742322.333	2687.21	0.154
2		1741069			
3		1747070			
4		1742728			
5		1742837			
6		1741069			

Table 6. Data for Intraday of Fostemsavir

Sr. No.	Concentration µg/ml	0 Hours	2 Hours	4 Hours	Mean	SD	%RSD
		Peak Area	Peak Area	Peak Area			
1	50	1735914	1726941	1735843	1732899	5160.19	0.297
2	60	2251353	2250086	2235834	2245758	8617.464	0.383
3	70	2664773	2666430	2638252	2656485	15811.96	0.595

Table 7. Data for Interday of Fostemsavir

Sr. No.	Concentration µg/ml	1 Day	2 Day	3 Day	Mean	SD	%RSD
		Peak Area	Peak Area	Peak Area			
1	50	1735914	1756713	1767573	1753400	16087.42	0.917
2	60	2251353	2252613	2287934	2263967	20765.88	0.917
3	70	2664773	2681386	2701649	2682603	18468.08	0.688

Table 8. Data for Percent Recovery by HPLC

Levels	% Mean recovery	SD	%RSD
50%	100	0.0311	0.0318
100%	100.2	0.0188	0.0188
150%	99.98	0.0124	0.0123

Table 9. Ruggedness Study of Fostemsavir

Sr.No.	Analyst	Conc.	Peak Area	Mean	SD	%RSD
1	Analyst 1	50	1729860	1728199	1771.83	0.102525
			1728403			
			1726334			
2	Analyst 2	50	1727484	1727843	990.1719	0.057307
			1727083			
			1728963			

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surface representation of the ideal formulation space is shown in Figure 5.

Linearity

The calibration curve for Fostemsavir, as shown in Figure 6, Figure 7 and Table 4, was linear over the 50-90 µg/ml concentration range. The calibration curve was graphed, and the regression equation was y = 33095x + 108199, yielding a correlation coefficient of 0.997.

Precision

Fostemsavir has a repeatability standard deviation (RSD) of less than 2% across six measurements at the same concentration (50 µg/ml). Table 5 displays data on the accuracy of repeat attempts. Table 6 and Table 7 displayed daily and hourly accuracy, respectively. If the % RSD is less than 2, then the created approach is accurate.

Accuracy

The recovery research was undertaken to establish the precision of the analytical method. Spiking levels of 50%, 100%, and 150% were used to prepare sample solutions. Table 8 displays the % recovery statistics acquired by the proposed HPLC technique. The % of recovery within 98-102% supports the established method's accuracy in accordance with the ICH Q2 (R1) requirements.

Ruggedness

Fostemsavir 50 µg/ml solution was utilised for ruggedness studies. The roughness was investigated as an extraneous influencing element by a change in analyst. By changing analysts, the % RSD for peak area was found to be less than 2 (Table 9).

Robustness

Fostemsavir 50 µg/ml solution was utilised for robustness studies. Modifying parameters inherent to the approach, also including mobile phase ratio, wavelength, column temperature, and flow rate, allows for careful examination of the robustness. By changing the ratio of mobile phase, wavelength, and flow rate, the % RSD for the peak region was found to be less than 2 (Table 10).

LOD and LOQ

Fostemsavir's LOQ and LOD were calculated to be 0.67 µg/ml and 0.22 µg/ml, respectively, using the standard deviation of the slope and intercept.

Solution stability

Fostemsavir's standard solution stability was analysed at 0, 24, and 48 hours. The data was reported as a percentage of unused medication. The acquired data demonstrated that the sample solutions were stable for a period of 48 hours. There is less than a 2% loss when kept at room temperature (Table 11).

Stability indicating a property

The analytical procedure's stability and specificity and the drug substance's degradation products can be determined. Fostemsavir solutions of 10 µg/ml were used in the degradation studies. Table 12 summarises the results of the forced deterioration studies.

Assay

After assaying Fostemsavir from tablets, the optimum chromatogram displayed a resolved peak with a retention period of 4.83 min. Fostemsavir label claim of % test of drug content was found to be 100.14 (n = 6). The assay result proved that the technique might be used to measure tablet powder despite excipients (Table 13) accurately.

Discussion

The analytically-sound layout Fostemsavir in pharmaceutical formulations may now be measured using an HPLC technique. Fostemsavir analysis by high-performance liquid chromatography relied on the retention duration, tailing factor, and theoretical plates to characterise the product profile. Analytical target product profiles are affected by many factors, but two, in particular, stand out as crucial quality attributes: mobile phase composition and flow rate. Using Design Expert Software Version 13.0, we implemented the central composite design for two factors over three layers. The risk assessment study identified key elements influencing the analytical target profile. During this chromatographic separation robustness investigation, we kept the column type, instrumentation, and injection volume the same while we varied the mobile phase concentration, flow rate, light wavelength, and column temperature.

The HPLC method for Fostemsavir was successfully developed using the quality-by-design strategy. Fostemsavir was measured using an RP-HPLC method that was optimised using a Shim-pack GIST C18 column (250 × 4.6 mm, 5µm particle size) with a mobile phase of acetonitrile and 1% formic acid (80:20 v/v). A constant rate of 0.8 ml/min was maintained.

A 266.0 nm UV detector was used to monitor the eluents. Fostemsavir had a retention time of 3.423 minutes. The approach had a linear correlation coefficient of 0.997 between 50 and 90 µg/ml. Repeatability, intraday, and interday precision were all determined to have a relative standard deviation (RSD) of less than 2%, showing the accuracy of the optimised approach. Both the LOD and LOQ were quite low, coming in at 0.22 and 0.67 µg/ml, respectively. According to the criteria established by the ICH guidelines, the percent recovery of spiked samples ranged from 99.57 ± 1.47 to 100.05 ± 1.73. The procedure was created using the ICH recommendations as a blueprint.

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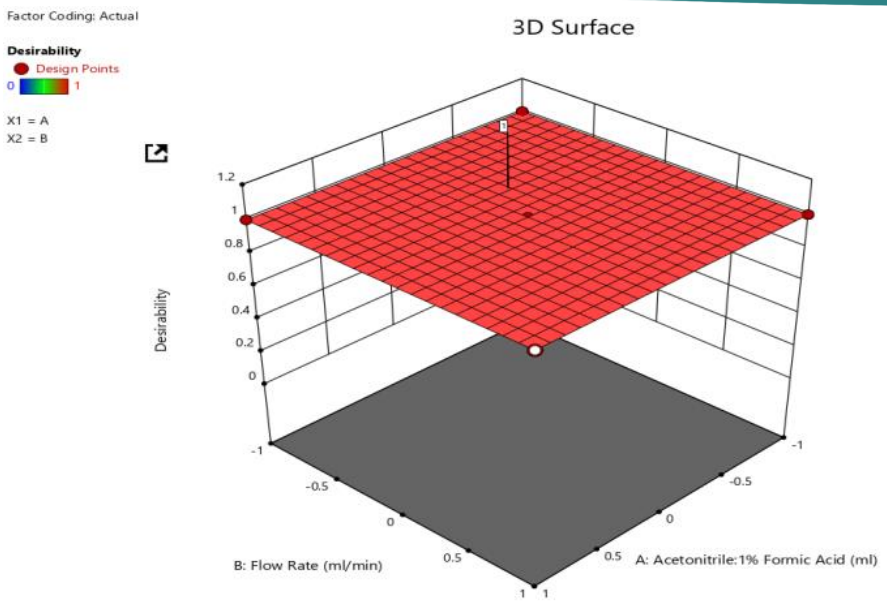

Figure 5. Optimised formulation's 3D surface plot of desirability

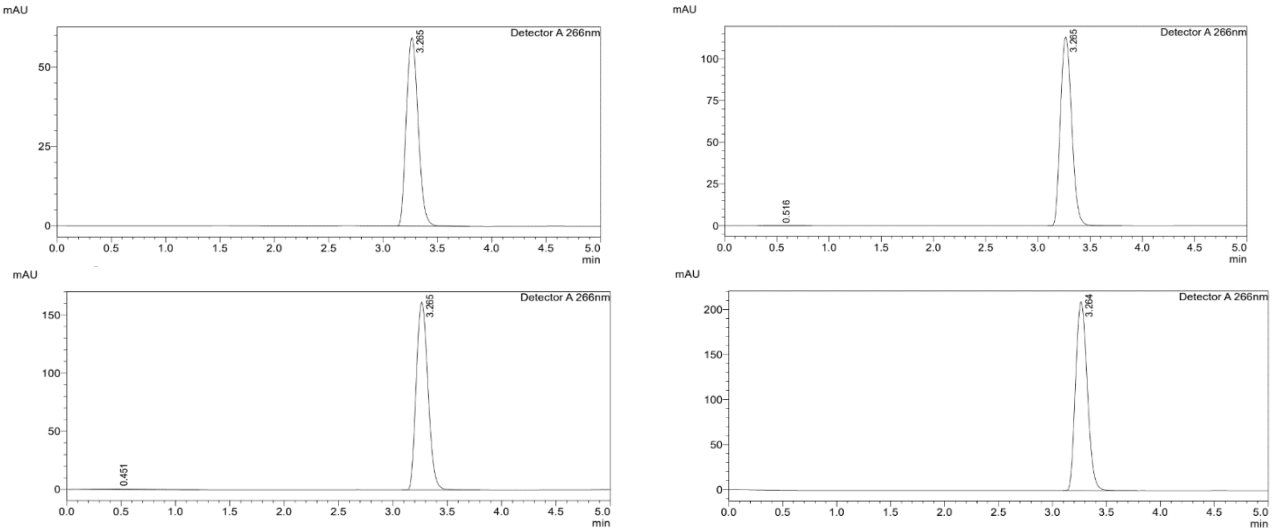

Figure 6. Linearity of 50-90 µg/ml Fostemsavir

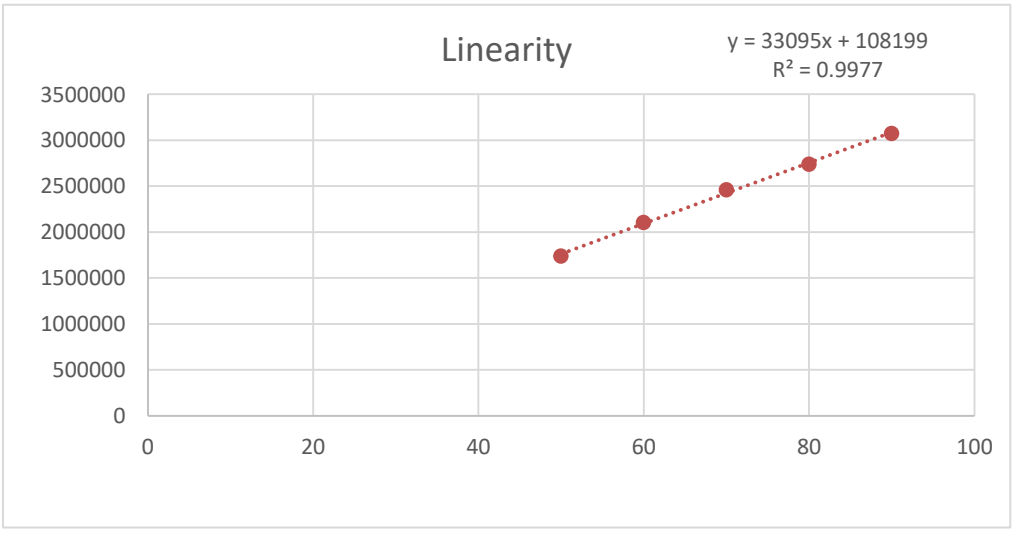

Figure 7. Linearity graph of Fostemsavir

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Table 10. Robustness study for Fostemsavir

Sr. No.	Parameter	Condition	Area	Mean	SD	%RSD
1	Change in Flow rate (ml/min)	0.6	1703525	1729920	23926.97	1.383
2		0.8	1736048			
3		1.0	1750187			
1	Change in Wavelength (nm)	205	1782249	1766516	28402.06	1.607
2		210	1733729			
3		215	1783570			
1	Change in Temperature (^{0}C)	35	1737962	1730698	6358.373	0.367
2		40	1726143			
3		45	1727988			
1	Change in Mobile phase ratio	70:30	1747997	1735695	11446.79	0.659
2		80:20	1733729			
3		90:10	1725358			

Table 11. Solution stability data

Time (h)	% Assay	%RSD
0	100.00	0.83
24	99.80	
48	98.48	

Table 12. Forced degradation studies for Fostemsavir

	Acid stress	Alkali stress	Peroxide stress	Thermal stress	Photolytic stress
% Recovered	91.20%	92.64%	92.85%	96.75%	98.05%
% Degradation	8.80%	7.36%	7.15%	3.25%	1.95%

Table 13. Assay results

Marketed formulation	Fostemsavir	
	Amount found	% Assay
RUKOBIA (Label claim)	600.86	100.34
	600.15	99.57
	600.56	100.28
	600.01	100.007
	597.16	98.58
	604.15	102.07
Mean	600.31	100.05
SD	2.30	1.14
% RSD	1.14	1.14

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Conclusion

In this work High Performance Liquid Chromatography (HPLC) guidelines have been provided, with a focus on assuring quality through methodical design. The analytical profile of the desired product determines the goals of the approach. The experimental design delineates the methodology by which the pivotal constituents of the High Performance Liquid Chromatography (HPLC) approach, namely the mobile phase and flow rate, can be evaluated.

A multivariate analysis was carried out using the principles of analytical quality by design (QbD) to determine the ideal system and ultimate design parameters for creating an HPLC approach for Fostemsavir.This included the interaction of two factors: the composition of the mobile phase and the flow rate at three different levels (Elder, 2013; Smith et al., 1999). Using a central composite design, we analysed and improved their interdependencies on multiple fronts. Here, we gain a deeper comprehension of the aspects affecting chromatographic separation in the efficacy of the procedures. This method provides a valuable experience that can be applied to the design of future chromatographic optimisation. All verified values fell within the range of allowable values. Linearity, precision, accuracy, specificity, robustness, and ruggedness were all seen in the validated method for determining Fostemsavir. There has been less room for error in method validation and transfer thanks to QbD's emphasis on thoroughly understanding all method variables. When compared to manual method creation, the time and effort required to create a high-quality QbD method utilising the Design Expert programme is significantly less. The data all indicate reproducibility, selectivity, accuracy, speed, and robustness. In perfecting the methodology, a previously unknown mobile phase was discovered. After being validated according to ICH requirements, the process will be incorporated into implementing standards for quality management in the pharmaceutical industry.

Conflict of Interest

There is no known conflict of interest in this manuscript.

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